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**APPLICATION FOR UNITED STATES LETTERS PATENT**

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Title: **METHOD OF MAKING A MULTI-WELL TEST PLATE  
HAVING ADHESIVELY SECURED TRANSPARENT  
BOTTOM PANEL**

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**SPECIFICATION**

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## **METHOD OF MAKING A MULTI-WELL TEST PLATE HAVING ADHESIVELY SECURED TRANSPARENT BOTTOM PANEL**

### **Cross-Reference to Related Applications**

This application is a divisional of Application No. 09/427,235, filed October 25, 1999, the disclosure of which is hereby fully incorporated by reference herein.

### **5     Field of the Invention**

The present invention generally relates to multi-well test plates or so-called micro-plates for assaying liquid samples and, more particularly, to multi-well test plates having glass bottoms secured to a framework of wells for containing the liquid samples.

### **10    Background of the Invention**

Multi-well test plates are well known in scientific areas, such as biotechnology, for allowing the detection and measurement of substances present in translucent liquid samples. Generally, this is accomplished by measuring the light absorbance characteristics of the sample through one or

more spectroscopy procedures. Typically, a framework of test wells is open at the top for receiving the liquid samples and is closed with a transparent bottom, formed of a polymer or glass, for allowing light radiation penetration in a wavelength region necessary for a particular study. These studies, commonly referred to assays, may include drug concentration assays, drug metabolite assays, enzyme activity assays, enzyme cofactor assays, fluorescent probe excitations or emissions, DNA spectral shifts or DNA and protein concentration measurements, as well as many other studies.

Due to the advantageous physical and optical properties of glass, glass bottom test plates can be more desirable than test plates having bottoms formed from a transparent polymer, such as polyolefins, fluoropolymers, polyesters, or other homopolymers and copolymers. The thickness of the transparent bottom has also been recognized as an important factor for achieving accurate test results. Moreover, when applying a glass sheet or panel to the bottom of a polymeric framework, for example, it has been difficult to achieve a seal in surrounding relation to each test well. Manufacturing methods involving an adhesive securement of the glass panel to the test well framework can be very slow and result in inadequate adhesion, inadequate sealing around the bottom of each well, adhesive migration into the test wells or other problems.

The present invention is therefore generally directed to the manufacture of multi-well test plates having an upper frame structure with multiple test wells and a thin, glass or other transparent bottom panel adhered to the framework in a fast and effective manner which does not have any adverse consequences related to the subsequent use of the test plate.

## **Summary of th Invention**

In one general aspect, the invention provides a multi-well test plate comprising an upper frame portion including a plurality of walls defining adjacent wells, each wall having an upper end and a lower end. The test plate  
5 further includes a transparent panel having an upper surface and a lower surface. The transparent panel is secured to the lower ends of the walls defining the wells by a layer of ultraviolet and visible (UV/V) light curable adhesive contacting the upper surface of the glass panel and the lower end of each wall surrounding a corresponding lower end of each well. The use of a  
10 UV/V curable adhesive allows the use of UV and visible light directed through the transparent panel to cure the adhesive between the transparent panel and upper frame portion. In the preferred embodiment, the transparent panel is glass; however, other transparent polymers or plastics may be used to form this panel while still realizing various benefits of this invention. The UV/V light  
15 curing process does not modify the glass bottom panel of the preferred embodiment in a manner that adversely affects performance.

As other equally important aspects of the invention, an adhesive as contemplated for the invention has various advantageous properties. These properties, singly or in various combinations, serve different functions and may  
20 be exhibited by a UV/V curable adhesive as mentioned above, or other heat curable or infrared curable adhesives or epoxies. For example, the adhesive has a viscosity that is preferably greater than about 8,000 cps to minimize flowing for more accurate adhesive placement. The adhesive is also thixotropic in nature in that it will not flow after application, for example, onto the upper  
25 surface of the glass panel. This has two beneficial results. First, the adhesive

will not flow into an area of the glass panel that will become the bottom surface of a well so as to potentially contaminate the samples within the well. Also, the adhesive will not thin out after application and consequently decrease the adhesion between the glass panel and the upper frame portion.

5                   The adhesive is also preferably transparent and this aspect has been found advantageous for the reason that it will not interfere with detection made from the bottom of the plate, due to specific wavelength absorbance or reflection. Curability by UV and visual light is advantageous because complete cure and nontoxicity is ensured by UV exposure followed by additional  
10 exposure to visual light. A one component adhesive as described herein eliminates the need for mixing and its associated problems such as variability in time between mixing and application. The adhesive cures to form a flexible joint to prevent stress cracking during cure and later during use of product at various temperatures. The adhesive is stable during and after ethylene oxide  
15 and gamma sterilization. The adhesive is nontoxic and is USP Class VI.

                  Moreover, the adhesive as used in this invention exhibits no off-gassing after curing. Such off-gassing could result in sample contamination. The adhesive is also water insoluble so as to be unaffected by the liquid samples contained in the test wells. The adhesive is non-autofluorescent so  
20 that the use of light during testing procedures or studies does not cause the adhesive to autofluoresce and adversely affect the study. The glass panel preferably has a thickness of about 0.006", as this thickness has been found to be most advantageous in the elimination of so-called cross talk and optimizes other optical properties. A suitable range of thicknesses for the glass panel  
25 may be from about 0.005" to about 0.040". Although various adhesives may be

used having one or more of the above-mentioned, advantageous properties, the preferred UV/V curable adhesive is an acrylated urethane adhesive or an adhesive with the same physical properties as claimed herein.

Additional objectives, advantages and features of the invention will become more readily apparent to those of ordinary skill in the art upon review of the following detailed description of the preferred embodiments, taken in conjunction with the accompanying drawings.

### **Brief Description of the Drawings**

Fig. 1 is a perspective view of a multi-well test plate constructed in accordance with one illustrative and preferred embodiment of the invention.

Fig. 2 is a top view of the multi-well test plate shown in Fig. 1.

Fig. 3 is a cross-sectional view taken along line 3-3 of Fig. 2.

Fig. 4 is a cross-sectional view schematically showing an initial step in a screen transfer process being used to initially apply adhesive to a screen during the manufacture of the test plate shown in Fig. 1.

Fig. 5 is a cross-sectional view similar to Fig. 4, but showing the adhesive transfer from the screen to the glass panel.

Fig. 6 is a top view of the glass panel after application of the adhesive through the screen as shown in Fig. 5.

Fig. 7 is a schematic, perspective view showing the upper frame portion being applied to the glass panel having the adhesive applied thereto as shown in Fig. 6.

#### **Detailed Description of the Preferred Embodiments**

Referring first to Figs. 1-3, a multi-well test plate 10 constructed in accordance with one preferred embodiment of the invention generally comprises an upper frame portion 12 having a plurality of intersecting walls 14, 16. Upper frame portion 12 is preferably formed from a polymer, such as polystyrene, and is preferably opaque. Depending on the intended use of test plate 10, upper frame portion 12 can be transparent, black or white in color. White has advantageous light reflective properties, while black has advantageous light absorptive properties. Walls 14, 16 form independent wells 18 for receiving liquid assay samples. The specific embodiment shown and described herein includes 384 square wells 18, however, it should be understood that a greater or fewer number of wells may be used in a multi-well test plate constructed in accordance with the inventive concepts. Also, the square wells shown and described herein are also for illustrative purposes and may be substituted with wells of various shapes, including circular wells or other polygonal-shaped wells. As best shown in Fig. 3, upper frame portion 12 is secured to a glass panel 20 by a layer of adhesive 22, as more specifically discussed below. Optionally, glass panel 20 may be substituted with a polymeric transparent panel suitable for the intended use of the product. It is important that the layer of adhesive 22 be positioned in surrounding relation to each well 18 between walls 14, 16 and glass panel 20. This must be accomplished while achieving sufficient adhesion between walls 14, 16 and glass panel 20, but without allowing adhesive to squeeze into wells 18 as glass panel 20 is applied to upper frame portion 12, as further described below.

The preferred adhesive 22 is an acrylated urethane, UV/V light curable adhesive. One preferred adhesive is Loctite® adhesive 3211, which exhibits all of the properties preferred in the present invention. These properties include UV/V light curability, thixotropic characteristics, transparency, no off-gassing after curing, water insolubility, non-autofluorescence, a viscosity greater than about 8,000 centipoise (cps), non-toxicity, the ability to release completely from an application screen and the affinity to transfer completely to a glass surface. Although an exaggerated thickness is shown in Fig. 3 for clarity, adhesive layer 22 is preferably applied in a thickness ranging from about 0.0005" to about 0.005". The preferred adhesive thickness is from about 0.002" to about 0.004". The thickness of glass panel 20 is preferably about 0.006", but may be within a range of thicknesses of about 0.005" to about 0.040". When the adhesive is applied, for example, in a grid pattern as described below, an edgewise gap of about 0.003" to about 0.005" is left on each side of the bottom of each wall 14, 16 so as to accommodate squeeze-out of the adhesive as glass panel 20 is applied to upper frame portion 12.

Turning now to Figs. 4-6, a schematic representation of an adhesive application process is shown using a screen 30 having apertures 32 formed into a grid pattern corresponding to the pattern of adhesive necessary to secure frame portion 12 to glass panel 20, as shown in Fig. 3. More specifically, adhesive 22 is initially applied in a generally even layer on screen 30 by a flood bar 33 (Fig. 4) and, in a subsequent step, a squeegee or doctor blade 34 (Fig. 5) is moved across the upper surface of screen 30 so as to force adhesive 22 through apertures 32 and onto the upper surface of glass panel 20. The thickness of adhesive layer 22 produced by flood bar 33 is preferably a



minimum thickness equal to the screen thickness. Screen 30 is preferably spaced about 1/16" from the upper surface of glass panel 20. During the adhesive application process, glass panel 20 is mounted on a suitable fixture 36, such as through the use of vacuum.

5                   As squeegee 34 is moved across screen 30, it pushes screen 30 against glass panel 20 with line contact as shown in Fig. 5. This squeezes adhesive 22 through apertures 32 leaving a grid pattern of adhesive on glass panel 20 as shown in Fig. 6. This pattern of adhesive includes intersecting lines of adhesive 22a, 22b corresponding respectively to intersecting walls 14,  
10   16 and a surrounding line of adhesive 22c for sealing around the outer periphery of glass panel 20 and a corresponding periphery upper frame portion 12. All lower surfaces of upper frame portion 12 are preferably molded with a mirror finish to facilitate uniform contact with adhesive 20. These lower surfaces may also be modified with a textured surface or other finish which  
15   increases surface area to enhance adhesive coverage.

                  As further shown in Fig. 7, upper frame portion 12 is applied to glass panel 20 while ensuring that the lower ends of intersecting walls 14, 16 are precisely aligned with the intersecting lines of adhesive 22a, 22b, 22c. Ultraviolet and visible light is then directed at the adhesive through glass panel  
20   20 to cure adhesive lines 22a, 22b, 22c. The cure is performed using an electrode or electrode-less lamp, such as a xenon lamp or a mercury vapor lamp. The lamp preferably provides light in both the visible and UV spectrums, i.e., in wavelengths ranging from 200 nm to 1,000 nm. The lamp is used in a power range of 500 to 1,000 watts, but this power may range from 200 to 2,000

watts. Depending on the power setting, the light is activated for a time ranging from about 2 seconds to about 30 seconds.

While the present invention has been illustrated by a detailed description of a preferred embodiment, it is not the intention of the applicants to  
5 restrict or in any way limit the scope of the appended claims to such detail. Various features of the invention may be combined in various unique and advantageous manners to achieve objectives of the invention. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details,  
10 representative apparatus and method, and illustrative example shown and described. Accordingly, departures may be made from such details without departing from the spirit or scope of applicants' general inventive concept.